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NOTES

Method for the Serological Typing of the Capsular Polysaccharides of *Staphylococcus aureus*

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A method is described for typing *Staphylococcus aureus* capsular polysaccharides that is based on direct bacterial cell agglutination and immunoprecipitation of cell extracts with monospecific antisera. Encapsulated strains were identified by their inagglutinability with teichoic acid antisera. The typing sera reacted specifically with extracts of eight prototype strains.

Staphylococcus aureus is the etiological agent of diverse clinical diseases. Classification of *S. aureus* by serological typing schemes based on agglutination reactions or growth characteristics in serum soft agar has been proposed (3, 4, 7, 8, 14, 17). Incomplete knowledge of the structural and pathogenic role of the structures involved in these reactions and the complexities of preparing standardized typing sera have limited the use of these methods. In the absence of a precise serological typing procedure for *S. aureus*, bacteriophage typing has been used (2).

Karakawa et al. (9–11) demonstrated that clinical isolates grown on appropriate media are encapsulated. These encapsulated strains are resistant to phagocytosis by polymorphonuclear leukocytes and produced extracellular polysaccharides different from teichoic acid. A classification scheme based on eight capsular antigens of *S. aureus* was reported, and it was found that about 70% of the blood isolates produce either the type 5 or type 8 capsular polysaccharides (1, 10). These data suggest that the pathogenesis of staphylococcal disease may be similar to that caused by other encapsulated bacteria. This typing scheme described for *S. aureus* is based on serologically defined capsular polysaccharides different from teichoic acid. There is increasing interest in identifying potential invasive *S. aureus* strains (16). Problems were encountered in detecting the capsular polysaccharide and in preparing typing antisera (5, 13, 15). For these reasons, we report in detail the preparation of capsular typing sera and the capsular typing of *S. aureus* strains.

Vaccines for the preparation of typing sera were prepared from cultures of prototype strains grown on Columbia medium (Difco Laboratories, Detroit, Mich.) agar supplemented with 2% sodium chloride (CSA medium) under an atmosphere of 5% CO_2 at 37°C for 18 h (1). The nonencapsulated teichoic acid-rich strain 32 was grown on tryptic soy agar (Difco). Cells were suspended with a glass rod in 20 ml of 3% Formalin in phosphate-buffered saline (PBS; pH 7.2) and incubated at room temperature for 18 h. The Formalin-treated cells were washed in PBS and suspended in 5% formalin–PBS to give an absorbance of 0.6 at 550 nm.

Vaccines were checked for viable organisms on CSA medium plates and for encapsulation by direct cell agglutination (see below) with teichoic acid antiserum (vide infra). Agglutination titers of less than 1:20 were considered to be indicative of encapsulation. New Zealand White rabbits,

TABLE 1. Agglutination of encapsulated and nonencapsulated *S. aureus* strains by capsular typing and teichoic acid antisera[a]

S. aureus strains	Antiserum	Reciprocal agglutination titer of:	
		Untreated cells	Acid-treated cells
Wood (nonencapsulated)	Teichoic acid	1,280	1,280
	Type 2 (K-93M)	640	640
	Type 5 (Reynolds)	640	640
	Type 8 (Becker)	640	320
K-93M (type 2)	Teichoic acid	<20	1,280
	Type 2	2,560	640
	Type 5	<20	640
	Type 8	<20	640
Reynolds (type 5)	Teichoic acid	<20	640
	Type 5	1,280	640
	Type 8	<20	320
Becker (type 8)	Teichoic acid	<20	320
	Type 8	640	320
	Type 5	<20	320

[a] Cells were grown on CSA medium and suspended in 0.5% formalinized PBS. A portion was treated with 0.2 M glycine-hydrochloride (pH 2.0) at 100°C for 10 min (acid treated) to remove the capsular polysaccharide.

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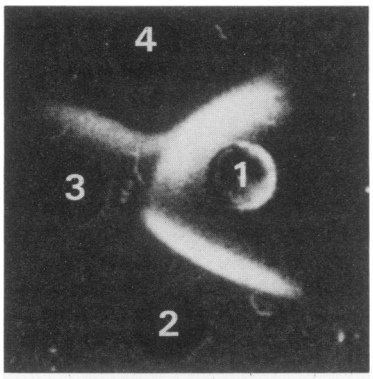

FIG. 1. Double immunodiffusion analysis of crude type 5 (strain Reynolds) antigen extract and of purified teichoic acid with unabsorbed and absorbed type 5 antiserum (strain Reynolds). Wells: 1, anti-type 5 (unabsorbed); 2, teichoic acid; 3, anti-type 5 (absorbed); 4, crude type 5 antigen extract.

weighing 6 lb (2.72 kg) each, were injected for 4 consecutive weeks, three times per week with 0.2 ml subcutaneously during week 1 and twice with 0.1 ml intravenously. Thereafter, only the intravenous route was used, and the dose was increased by 0.1 ml each week. A sample of serum was tested 5 days after injection by agglutination with the homologous vaccines. Sera were collected when agglutination titers reached ≥1,280. The nonencapsulated strain 57 used for absorbing noncapsular antibodies from this antisera was grown in 1 liter of tryptic soy broth (Difco) for 18 h at 37°C. The bacteria were killed by heating at 70°C for 4 h, centrifuged at 10,000 × *g*, and suspended in 100 ml of PBS. The resulting suspension was treated with 100 mg of trypsin (3× crystallized; Sigma Chemical Co., St. Louis, Mo.) and incubated at 37°C for 8 h with a few drops of chloroform. The treated cells were washed three times with PBS. Rabbit antisera were absorbed by adding 2 volumes of serum to 1 volume of packed cells and then dispersed by gently stirring.

After 18 h at 4°C, cells were removed by centrifugation at 10,000 × *g*, and the serum was stored with 0.02% sodium azide at 4°C. Residual teichoic acid antibodies in absorbed typing sera were measured by direct cell agglutination.

S. aureus produce several surface antigens, including capsular polysaccharides and teichoic acids. These antigens can be readily demonstrated by direct cell agglutination with specific sera. Cell suspensions prepared as described above for the preparation of vaccines were added (0.25 ml) to twofold dilutions of test sera (0.25 ml) made in PBS. The mixtures were incubated for 4 h at 37°C, and the titer was recorded as the highest dilution yielding macroscopic agglutination. A nonencapsulated strain of *S. aureus*, strain Wood, was agglutinated by both teichoic acid antiserum and heterologous unabsorbed typing sera (Table 1). Encapsulated strains, prototypes 2, 5, and 8, gave agglutination reactions with only homologous typing serum; these strains did not react with teichoic acid antiserum. These results suggest that the teichoic acid of prototype encapsulated strains is not exposed and that the Wood strain is devoid of capsular antigens. To support this notion, the agglutination of both encapsulated and nonencapsulated strains was compared before and after removal of capsular antigen by acid. Cell suspensions were treated with 0.2 M glycine-hydrochloride buffer (pH 2.0) to remove the capsular antigen. Packed cells (1 ml) were suspended in 5 ml of the pH 2.0 buffer and boiled for 10 min (12). The cell suspension was adjusted to pH 7.2 with 0.1 N NaOH and washed twice with PBS by centrifuging for 15 min at 12,000 × *g* at 4°C. After the removal of capsular antigens by acid treatment (Table 1), the encapsulated strains were strongly agglutinated by teichoic acid antiserum. In contrast, treatment of the Wood strain with acid did not affect its strong agglutination with teichoic acid antiserum.

These results suggest that the inagglutinability of *S. aureus* strains in the presence of teichoic acid antiserum can be used as a presumptive test for encapsulation of *S. aureus*.

Unabsorbed sera showed evidence of heterologous reactions (Table 1) with unencapsulated strains. Specific typing sera prepared to the eight known encapsulated prototype strains of *S. aureus* were absorbed with the nonencapsulated trypsinized strain 57. This absorption procedure was shown to be effective in removing the major cross-reactive antibody in *S. aureus* antisera. In a representative double-immunodiffusion test (Fig. 1), crude extract of type 5 formed multiple precipitin bands with homologous unabsorbed serum but only a single precipitin band with absorbed serum. In contrast, purified teichoic acid (6) reacted with unabsorbed serum but not with the absorbed type 5 serum. These results indicate that absorbed type 5 serum contains type 5 capsular antibodies and is devoid of anti-teichoic acid antibodies. Typing sera prepared against eight prototype encapsulated strains were subsequently tested by the direct cell agglutination procedure and shown to be type specific (Table 2). The nonencapsulated strain Wood gave an agglutination titer of 1,280 with teichoic acid antiserum and <20 with typing sera. In contrast, the prototype strains were agglutinated by homologous sera at dilutions of >600 and <20 by teichoic acid antiserum. Strains 7007 and Reynolds were agglutinated by antisera prepared against both strains. Thus, there ap-

TABLE 2. Agglutination reactions between prototype strains and homologous and heterologous typing sera and teichoic acid antiserum

Prototype strains (capsular type)	Reciprocal agglutination titer of typing antiserum								
	1	2	3	4	5	6	7	8	TA[a]
Wood (nonencapsulated)	<20	<20	<20	<20	<20	<20	<20	<20	1,280
Dp (1)	1,280	<20	<20	<20	<20	<20	<20	<20	<20
11127-var (2)	<20	2,560	<20	<20	<20	<20	<20	<20	<20
Mardi (3)	<20	<20	640	<20	<20	<20	<20	<20	<20
7007 (4)	<20	<20	<20	1,280	320	<20	<20	<20	<20
Reynolds (5)	<20	<20	<20	640	1,280	<20	<20	<20	<20
C (6)	<20	<20	<20	<20	<20	640	<20	<20	<20
207 (7)	<20	<20	<20	<20	<20	<20	640	<20	<20
Becker (8)	<20	<20	<20	<20	<20	<20	<20	640	<20

[a] Reciprocal of the highest dilution which gave a 2+ reaction.

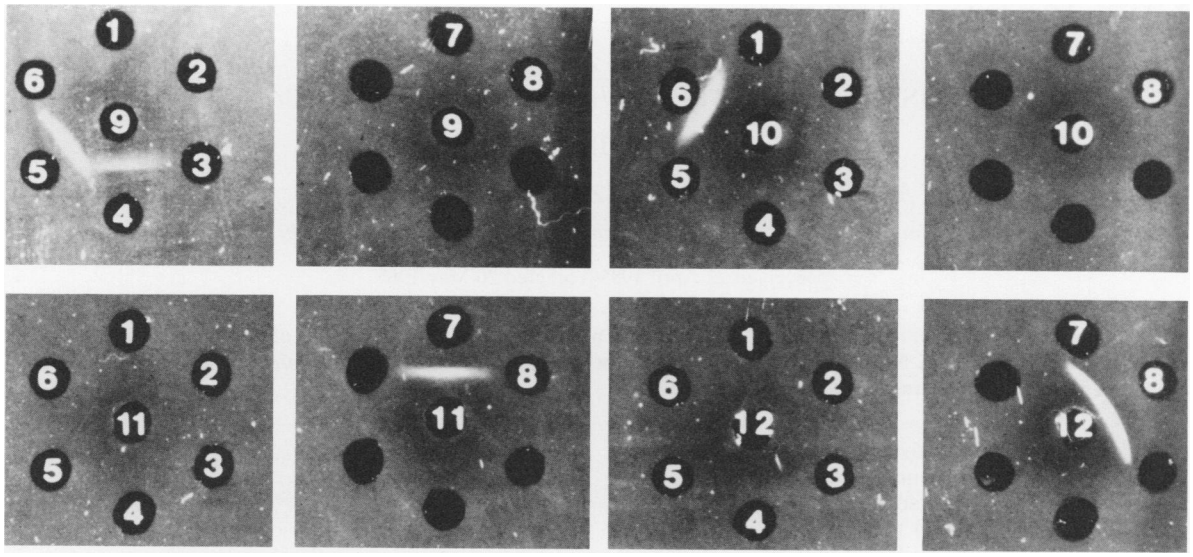

FIG. 2. Double-immunodiffusion analysis of eight capsular polysaccharides with absorbed serotype serum 5, 6, 7, and 8. Wells 1 to 8 represent each of the eight known serotype antigens. Wells: 9, absorbed type 5 serum; 10, absorbed type 6 serum; 11, absorbed type 7 serum; 12 absorbed type 8 serum.

pears to be a cross-reaction between antigens of type 4 and type 5 strains.

The double-immunodiffusion test on the eight typing sera confirmed their type specificity. Each typing serum gave a single precipitin band with its homologous crude extract of capsular antigen (Fig. 2). Again, a cross-reaction between types 4 and 5 was noted.

These methods have been reproducible and have used prototype strains producing serologically defined capsular polysaccharides. The proposed typing scheme relies upon the cultivation of encapsulated isolates of *S. aureus*, the preparation of specific capsular antisera, and capsular antigen free of contaminating teichoic acids.

The nomenclature which has been put forth is not final since other serotypes probably exist. The proposed method is useful for differentiating clinical isolates according to their capsular polysaccharides, which may be virulence structures and protective antigens. This typing scheme has been applied to selected isolates of *S. aureus* to determine the effectiveness of the scheme in identifying prevalent serotype strains of *S. aureus*. Arbeit et al. (1) demonstrated the prevalence of types 5 and 8 in a limited number of hospital isolates. This method can potentially complement existing methods such as antibiograms and phage typing for clinical and epidemiological studies.

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